(FILE 'HOME' ENTERED AT 14:24:16 ON 03 OCT 2001)

FILE 'USPATFULL' ENTERED AT 14:24:34 ON 03 OCT 2001
L1 0 S (END SEQUENCE PROFILING)
L2 11 S (GENOME (8A) REFERENCE)/CLM
L3 4 S (GENOME (8A) REFERENCE)/CLM NOT AMPLIF?/CLM
L4 8254 S (NUCLEOTID? OR POLYNUCLEOTID? (8A) REFERENCE)/CLM NOT AMPLIF?
L5 157 S GENOME AND (SEQUENC### AND REFERENCE?)/CLM NOT AMPLIF?/CLM
L6 48 S L5 AND (TERMIN? OR END)/CLM
L7 26 S L6 AND METHOD/CLM
L8 19 S L5 AND TERMIN###/CLM

=> d l7 1,5,8,10,12,14,20,22 bib, clm

L7 ANSWER 1 OF 26 USPATFULL
AN 2001:167898 USPATFULL
TI Method for detecting and identifying mutations
IN Stefano, James E., Hopkinton, MA, United States
PA Genzyme Corporation, Framingham, MA, United States (U.S. corporation)
PI US 6297010 B1 20011002
AI US 1998-16542 19980130 (9)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Arthur, Lisa B.; Assistant Examiner: Souaya, Jehanne
LREP Konski, Antoinette F., Dugan, Deborah A.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 1351
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** for identifying one or more genetic alterations in a sample polynucleotide strand, comprising: (a) providing a duplex comprising the sample polynucleotide strand and a **reference** polynucleotide strand; (b) contacting the duplex with an agent which recognizes and protects base pair mismatches under conditions which allow the agent to bind to and protect the duplex at the mismatch to form a duplex:agent complex; (c) removing unprotected base pairs; (d) providing a preselected site from which to **sequence**; and (e) determining the **sequence** of the sample strand to identify the one or more genetic alterations in the sample polynucleotide strand.

2. The **method** according to claim 1, wherein step (d) comprises contacting the duplex with an agent having DNA polymerase activity and a mixture of 2 to 3 different deoxynucleoside triphosphates.

3. The **method** according to claim 1, wherein step (d) comprises ligating an adapter oligonucleotide to the sample stand.

4. The **method** according to claim 1 which further comprises step (a)(i), which comprises immobilizing the duplex to a solid support.

5. A **method** for identifying one or more genetic alterations in a sample polynucleotide strand, comprising: (a) providing a plurality of duplexes, wherein each duplex comprises a sample polynucleotide strand and a **reference** polynucleotide strand; (b) contacting the duplexes with an agent which recognizes and protects base pair mismatches under conditions which allow the agent to bind to and protect the duplexes at the mismatch to form duplex:agent complexes; (c) removing unprotected base pairs; (d) providing a preselected site from which to **sequence**; and (e) determining the **sequence** of the sample strands to identify the one or more genetic alterations in the sample polynucleotide strands.

6. The **method** according to claim 5, wherein step (d) comprises contacting the duplexes with an agent having DNA polymerase activity and a mixture of 2 to 3 different deoxynucleoside triphosphates.

7. The **method** according to claim 5, wherein step (d) comprises ligating an adapter oligonucleotide to the sample stands.

8. The **method** according to claim 5, which further comprises step (a)(i), which comprises immobilizing the duplex to a solid support.

9. A **method** for identifying one or more genetic alteration(s) in a sample nucleotide strand comprising: (a) providing a duplex by immobilizing the sample polynucleotide strand on one or more solid supports and contacting the sample polynucleotide strand with a **reference** polynucleotide strand under conditions suitable to form a duplex between the sample and **reference** strands; (b) contacting the duplex with an agent which recognizes and protects base pair mismatches under conditions which allow the agent to bind to and protect the duplex at the mismatch to form a duplex:agent complex; (c) removing unprotected base pairs; (d) providing a preselected site from which to **sequence**; and (e) **sequencing** the sample polynucleotide strands to identify the one or more genetic alteration(s).

10. The **method** according to claim 9, wherein step (d) comprises contacting the duplex with an agent having DNA polymerase activity and a mixture of 2 to 3 different deoxynucleoside triphosphates.

11. The **method** according to claim 9, wherein step (d) comprises ligating an adapter oligonucleotide to the sample stand.

12. A **method** for identifying one or more genetic alteration(s) in one or more sample polynucleotide strands comprising: (a) providing a plurality of duplexes by immobilizing a plurality of sample polynucleotide strands on one or more solid supports and contacting the plurality of sample polynucleotide strands with a **reference** polynucleotide strand under conditions suitable to form a duplex between the sample and **reference** strands; (b) contacting the duplex with an agent which recognizes and protects base pair mismatches under conditions which allow the agent to bind to and protect the duplex at the mismatch to form a duplex:agent complex; (c) removing unprotected base pairs; (d) providing a preselected site from which to **sequence**; and **sequencing** the sample polynucleotide strands to identify the alteration(s).

13. The **method** according to claim 12, wherein step (d) comprises contacting the duplex with an agent having DNA polymerase activity and a mixture of 2 to 3 different deoxynucleoside triphosphates.

14. The **method** according to claim 12, wherein step (d) comprises ligating an adapter oligonucleotide to the sample stand.

15. The **method** according to any of claims 1, 5, 9 or 12, wherein step (d) comprises ligating two adapter oligonucleotides to the product(s) of step (c).

16. The **method** according to claim 15, wherein the two adapter oligonucleotides are single-stranded.

17. The **method** according to claim 15, wherein following ligation of the adapter oligonucleotides, any remaining strands are degraded and extended upon the adapter template to produce a double-stranded products.

18. The **method** according to any of claims 1, 5, 9 or 12, wherein the agent is MutS.

19. The **method** according to any of claims 1, 5, 9 or 12,

wherein the **reference** strand further comprises a biotin or analog thereof at the 5' t**ini**.

20. The **method** according to any of claims 1, 5, 9 or 12, wherein the **reference** strand is selected from the group consisting of a PCR product, a multiplex restriction product, a cDNA, and a mRNA.

21. The **method** according to any of claims 1, 5, 9 or 12, wherein the sample strand is selected from the group consisting of a PCR product, a multiplex restriction product, a cDNA, and a mRNA.

L7 ANSWER 5 OF 26 USPATFULL
AN 2001:63455 USPATFULL
TI Method for characterizing DNA sequences
IN Schmidt, Gunter, Cambridge, United Kingdom
Thompson, Andrew Hugin, Ayr, United Kingdom
PA Brax Genomics Limited, Cambridge, United Kingdom (non-U.S. corporation)
PI US 6225077 B1 20010501
WO 9810095 19980312
AI US 1999-254023 19990420 (9)
WO 1997-GB2403 19970905
19990420 PCT 371 date
19990420 PCT 102(e) date
PRAI GB 1996-18544 19960905
DT Utility
FS Granted
EXNAM Primary Examiner: Patterson, Jr., Charles L.
LREP Burns, Doane, Swecker & Mathis, L.L.P.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 1501
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** for characterizing CDNA, which comprises: (a) cutting a sample comprising a population of one or more cDNAs or isolated fragments thereof, each having a strand complementary to the 3' poly-A **terminus** of an mRNA and bearing a tail, with a first sampling endonuclease at a first sampling site of known displacement from a **reference** site proximal to the tail to generate from each cDNA or isolated fragment thereof a first and second sub-fragment, each comprising a sticky **end sequence** of predetermined length and unknown **sequence**, the first sub-fragment bearing the tail; (b) sorting either the first or second sub-fragments into sub-populations according to their sticky **end sequence** and recording the sticky **end sequence** of each sub-population as the.first sticky **end**; (c) cutting the sub-fragments in each sub-population with a second sampling endonuclease, which is the same as or different from the first sampling endonuclease, at a second sampling site of known displacement from the first sampling site to generate from each sub-fragment a further sub-fragment comprising a second sticky **end sequence** of predetermined length and unknown **sequence**; and (d) determining each second sticky **end sequence**; wherein the aggregate length of the first and second sticky **end sequences** of each sub-fragment is from 6 to 10; and wherein the **sequences** and relative positions of the **reference** site and first and second sticky **ends** are utilized to characterize the cDNA or cPNAs.

2. A **method** according to claim 1, wherein the sample cut with the first sampling endonuclease comprises isolated fragments of the cDNAs produced by cutting a sample comprising a population of one or more cDNAs with a restriction endonuclease and isolating fragments whose restriction site is at the **reference** site.

3. A **method** according to claim 2, wherein the first sampling endonuclease binds to a first recognition site and cuts at the first sampling site at a predetermined displacement from the restriction site of the restriction endonuclease.

4. A **method** according to claim 3, wherein the first recognition site is provided in a first adaptor oligonucleotide which is hybridried to the restriction site of the isolated fragments.

5. A **method** according to claim 2, wherein the restriction endonuclease recognizes a 4 base pair binding site.

6. A **method** according to claim 2, wherein the second sub-fragments are sorted in step (b).

7. A **method** according to claim 1, wherein the first sampling endonuclease binds to the **reference** site and cuts at the first sampling site at a predetermined displacement from the **reference** site.

8. A **method** according to claim 1, wherein the first sampling endonuclease comprises a Type IIs endonuclease.

9. A **method** according to claim 1, wherein the second sampling endonuclease binds to a second recognition site and cuts at the second sampling site at a predetermined displacement from the first sampling site.

10. A **method** according to claim 9, wherein the second sampling endonuclease comprises a Type IIs endonuclease.

11. A **method** according to claim 9, wherein the second recognition site is provided in a second adaptor oligonucleotide which is hybridized to the first sticky **end**.

12. A **method** according to claim 1, wherein the tails of the cDNAs or fragments thereof are bound to a solid phase matrix.

13. A **method** according to claim 1, wherein the aggregate length of the first and second sticky **end sequences** of each sub-fragment is 8.

14. A **method** according to claim 13, wherein the length of each sticky **end** is 4.

15. A **method** according to claim 1, wherein the step (b) of sorting the sub-fragments comprises dividing the sub-fragments into an array of samples, each sample in a separate container; contacting the array of samples with an array of solid phase affinity matrices, each solid phase affinity matrix bearing a unique base **sequence** of the same predetermined length as the first sticky **end**, so that each sample is contacted with one of the possible base **sequences** and the array of samples is contacted with all possible base **sequences** of that predetermined length for hybridization to occur only between each unique base **sequence** and first sticky **end** complementary with one another; and washing unhybridized material from the containers.

16. A **method** according to claim 1, wherein the step (d) of determining each second sticky **end sequence** comprises isolating the further sub-fragments from step (c) and contacting the further sub-fragments with an array of adaptor oligonucleotides in a cycle, each adaptor oligonucleotide bearing a label and a unique base **sequence** of the same predetermined length as the second sticky **end**, the array containing all possible base **sequences** of that predetermined length; wherein

the cycle comprises sequentially contacting each adaptor oligonucleotide of the array with each sub[redacted]pulation of isolated sub-fragm[redacted]s under hybridization conditions, removing any unhybridized adaptor oligonucleotide and determining the presence of any hybridized adaptor oligonucleotide by detection of the label, then repeating the cycle, until all of the adaptors in the array have been tested.

17. A **method** according to claim 1, wherein the step (b) of sorting the sub-fragments comprises (i) binding the sub-fragments to a hybridization array comprising an array of oligonucleotide sets, each set bearing a unique base **sequence** of the same predetermined length as the first sticky **end** and identifiable by location in the array, all possible base **sequences** of that predetermined length being present in the array, so that each sub-population bearing its unique first sticky **end** is hybridized at an identifiable location in the array; and (ii) determining the location to identify the first sticky **end sequence**.

18. A **method** according to claim 1, wherein the sub-fragments cut in step (c) are those bound to the hybridization array so that the further sub-fragments generated thereby remain bound to the hybridization array; and wherein the step (d) of determining each second sticky **end sequence** comprises contacting the further sub-fragments under hybridization conditions with an array of adaptor oligonucleotides, each adaptor oligonucleotide bearing a label and a unique base **sequence** of the same predetermined length as the second sticky **end**, the array containing all possible base **sequences** of that predetermined length, removing any unhybridized adaptor oligonucleotide, and determining the location of any hybridized adaptor oligonucleotide by detection of the label.

19. A **method** for identifying cDNA in a sample, which comprises characterizing cDNA in accordance with a **method** according to any one of the preceding claims, comparing the **sequences** and relative positions of the **reference** site and first and second sticky **ends** obtained thereby with the **sequences** and relative positions of the **reference** site and first and second sticky **ends** of known cDNAs in order to identify each CDNA in the sample.

20. A **method** for assaying for one or more specific cDNAs in a sample, which comprises performing a **method** according to claim 1, wherein the **reference** site is predetermined, each first sticky **end sequence** in sorting step (b) is a predetermined first sticky **end sequence**, each second sticky **sequence** in step (d) is determined by assaying for a predetermined second sticky **end sequence**, and the relative positions of the **reference** site and predetermined first and second sticky **ends** characterize the or each specific cDNA.

21. A **method** according to claim 20, wherein the **reference** site and first and second sticky **end sequences** are predetermined by selecting corresponding **sequences** from one or more known target cDNAs.

L7 ANSWER 8 OF 26 USPATFULL
AN 2000:91705 USPATFULL
TI Automated DNA sequencing comparing predicted and actual measurements
IN Collinge, John, London, United Kingdom
Thornley, David, Perivale, United Kingdom
PA Imperial College of Science, Technology and Medicine, London, United Kingdom (non-U.S. corporation)
PI US 6090550 20000718
WO 9620286 19960704
AI US 1997-860050 19970828 (8)

WO 1995-GB3026 19951222
19970828 PCT 371 date
19970828 PCT 102(e) date
PRAI GB 1994-26223 19941223
GB 1995-3526 19950222
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Nixon & Vanderhye P.C.
CLMN Number of Claims: 46
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1084
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A **method** of automatically **sequencing** a DNA strand, comprising: (a) experimentally determining, for each position in the strand, a measurement representative of a base at that position; and (b) starting with an initial **sequence** comprising a part of the strand where the bases are assumed known, repeatedly building bases onto a growing **sequence**; and at each step determining a new base to add to a new position in the growing **sequence** in dependence upon both the measurement at the new position and upon at least some of the previously-determined bases in the growing **sequence**; the **method** including at each step, predicting the measurement at the new position, comparing the predicted measurement with the actual measurement at the new position, and determining the new base as a result of the comparison.

2. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the predicted measurement for the new position is calculated using a fixed number of the previously-determined bases in the growing **sequence**.

3. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the predicted measurement for the new position is calculated using at least some of the previously-determined bases in the growing **sequence**.

4. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the predicted measurement for the new position is calculated without **reference** to the measurements for any position in the strand.

5. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the predicted measurement for the new position comprises four separate values, one for each possible base.

6. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which said measurement at each position comprises four separate values, one for each possible base at that position.

7. A **method** of automatically **sequencing** a DNA strand as claimed in claim 6 in which a base is rejected as a candidate for the new position if its actual value for that position is less than an expected minimum value, the expected minimum value being calculated as a function of the predicted measurement for that base at that position.

8. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the growing **sequence** is created base by base, with the new base to be added being next in the **sequence** to the last previous base added.

9. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the new base to be added to the growing **sequence** is not adjacent in the **sequence** to the last

previous base added.

10. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the growing **sequence** grows in both directions along the strand from the initial **sequence**.

11. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 including simultaneously growing a plurality of growing **sequences** from a starting plurality of initial **sequences**.

12. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 including at a given step, determining the new base for said given step at least partially in dependence upon a preferred hypothetical base, said preferred hypothetical base being determined by looking ahead one step beyond said given step.

13. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 including at a given step looking ahead a plurality of steps, hypothesising a plurality of possible base **sequences**, and determining the new base for the given step at least partially in dependence upon a preferred hypothesised base **sequence**.

14. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which, at each step, an error measure is constructed based upon the predicted measurement and the actual measurement at the new position, accumulative error measure being kept for at least a part of the growing **sequence**, and the new base being determined according to the particular base that minimises, the accumulative error measure.

15. A **method** of automatically **sequencing** a DNA strand as claimed in claim 12 in which, at each step, an error measure is constructed based upon the predicted measurement and the actual measurement at the new position, accumulative error measure being kept for at least a part of the growing **sequence**, and the new base being determined according to the particular base that minimises the accumulative error measure, in which the preferred hypothetical base is determined according to the particular base that minimises the accumulative error measure.

16. A **method** of automatically **sequencing** a DNA strand as claimed in claim 12 in which, at each step, an error measure is constructed based upon the predicted measurement and the actual measurement at the new position, accumulative error measure being kept for at least a part of the growing **sequence**, and the new base being determined according to the particular base that minimises the accumulative error measure, and in which the preferred hypothetical base **sequence** is determined according to the particular **sequence** that minimises the accumulative error measure.

17. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the Sanger technique is used to experimentally determine, for each position in the strand, the measurement representative of the base at that position.

18. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the measurements are obtained using a modified Sanger technique in which the reaction **terminators** are each individually labelled according to their respective bases, and in which all are mixed within a single reaction volume.

19. A **method** of automatically **sequencing** a DNA strand as claimed in claim 18 in which the reaction primer is also

labelled, the information from the primer labels being used to normalise the **terminator** label measurements.

20. A **method** of automatically **sequencing** a DNA strand as claimed in claim 1 in which the predicted measurement for the new position is calculated using a mathematical model or a look-up table which simulates the replication effect.

21. A **method** of automatically **sequencing** a DNA strand as claimed in claim 18 in which the reaction **terminators** are dye-labelled.

22. A **method** of automatically **sequencing** a DNA strand as claimed in claim 21 in which the predicted measurement for the new position is calculated using a mathematical model or a look-up table which simulates the fluorescence effect.

23. A **method** of automatically **sequencing** a DNA strand as claimed in claim 17 in which the primer is dye-labelled.

24. A **method** of determining the characteristics of a fetus of a pregnant female comprising obtaining a sample from the female, the sample including fetal cells, and automatically **sequencing** a DNA strand derived from the fetal cells using a **method** as claimed in claim 1.

25. A **method** as claimed in claim 24 in which the sample is a blood sample.

26. A **method** as claimed in claim 25 in which the sample is a sample of the venous blood of the pregnant female.

27. A **method** as claimed in claim 24 in which the sample is a mucus sample.

28. A **method** as claimed in claim 27 in which the sample is a cervical mucus sample.

29. A **method** as claimed in claim 24 including the step of concentrating the fetal DNA in the sample prior to **sequencing**.

30. A **method** as claimed in claim 29 including the step of concentrating the fetal cells in the sample.

31. A **method** as claimed in claim 30 in which the fetal cells are concentrated by binding them using a cell-specific antibody.

32. A **method** as claimed in claim 24 in which the determining of the characteristics comprises detecting chromosomal abnormalities.

33. A **method** as claimed in claim 24 in which the determining of the characteristics comprises detecting DNA mutations.

34. A **method** of detecting a pathogen in a human or animal patient comprising obtaining a sample from the patient, the sample including the pathogen, and automatically **sequencing** a DNA strand derived from the pathogen using a **method** as claimed in claim 1.

35. A **method** as claimed in claim 34 including the step of determining the quantity of pathogen present by measuring the load of pathogen DNA in the sample.

36. A **method** as claimed in claim 34 in which the sample is a blood sample.

37. A **method** as claimed in claim 34 in which the sample is a

mucus sample.

38. A **method** as claimed in claim 34 in which the sample is a urine sample.

39. A **method** as claimed in claim 34 in which the sample is a semen sample.

40. A **method** as claimed in claim 34 including the step of concentrating the pathogen DNA in the sample prior to **sequencing**.

41. A **method** as claimed in claim 35 in which the load of pathogen DNA is determined as a proportion of the total sample DNA.

42. A **method** of detecting foreign DNA in a body sample, comprising **sequencing** DNA strands in the sample using a **method** as claimed in claim 1 and determining whether foreign DNA is present by comparing the **sequenced** DNA strands from the sample with **sequenced** DNA strands derived from a further body sample known to have no foreign DNA.

43. A **method** of detecting heterozygous **sequences**, comprising **sequencing** a pair of DNA strands using a **method** as claimed in claim 1, at each step simultaneously determining the base pairs to be added to the corresponding positions in the growing **sequences**.

44. A **method** of automatically **sequencing** a mixture of separate DNA strands of a first type and a second type, comprising **sequencing** the separate strands using a **method** as claimed in claim 1, at each step determining the base allocations to be added to the corresponding new positions in the growing **sequences**.

45. A **method** as claimed in claim 44 including determining the relative proportions of DNA of the first type and of the second type.

46. A **method** of determining the relative proportions of a first body sample and a second body sample in an admixed sample, the **method** comprising **sequencing** DNA strands in the admixed sample using a **method** as claimed in claim 1 determining the relative proportions of DNA from the first sample and from the second sample, and determining the relative proportions of the body samples from the relative proportions of DNA.

L7 ANSWER 10 OF 26 USPATFULL
AN 2000:43929 USPATFULL
TI Method for identifying variations in polynucleotide sequences
IN Murphy, Patricia D., Slingerlands, NY, United States
White, Marga B., Frederick, MD, United States
PA Gene Logic, Inc., Gaithersburg, MD, United States (U.S. corporation)
PI US 6048689 20000411
AI US 1997-825487 19970328 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.; Assistant Examiner: Taylor, Janell E.
LREP Halluin, Albert P.Howrey Simon Arnold & White, LLP.
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 3451
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A **method** for determining the presence or absence of a

sequence variation in a gene sample, comprising the sequential steps of: (a) performing an allele specific hybridization assay for the presence or absence of one or more pre-determined **sequence** variations; (b) if no pre-determined **sequence** variation is found in step (a), then performing a **sequence** variation locating assay selected from the group consisting of protein truncation assay, single strand conformation polymorphism analysis, heteroduplex analysis, denaturing gradient gel electrophoresis, constant denaturant gel electrophoresis, and chemical cleavage analysis; (c) if no **sequence** variation is found in step (b), then **sequencing** the gene sample; and (d) determining the presence or absence of a **sequence** variation by analyzing the **sequence**(s) obtained in step (c) against a **reference sequence**.

2. A **Method** for determining the presence or absence of a **sequence** variation in a gene sample, comprising the sequential steps of: (a) performing an allele specific hybridization assay for the presence of one or more pre-determined **sequence** variations; (b) if no pre-determined **sequence** variation is found in step (a), then performing a **sequence** variation locating assay selected from the group consisting of protein truncation assay, single strand conformation polymorphism analysis, heteroduplex analysis, denaturing gradient gel electrophoresis, constant denaturant gel electrophoresis, and chemical cleavage analysis; (c) if a **sequence** variation is detected in step (b), then performing targeted confirmatory **sequencing**; and (d) determining the presence or absence of a **sequence** variation by analyzing the **sequence**(s) obtained in step (c) against a **reference sequence**.

3. A **method** for determining the presence or absence of a **sequence** variation in a gene sample, comprising the sequential steps of: (a) performing an allele specific hybridization assay for the presence or absence of one or more pre-determined **sequence** variations; and (b) if no pre-determined **sequence** variation is found in step (a), then **sequencing** the gene sample; and (c) determining the presence or absence of a **sequence** variation by analyzing the **sequence**(s) obtained in step (b) against a **reference sequence**.

4. A **method** for determining the presence or absence of a **sequence** variation in a gene sample, comprising the sequential steps of: (a) performing a **sequence** variation locating assay selected from the group consisting of protein truncation assay, single strand conformation polymorphism analysis, heteroduplex analysis, denaturing gradient gel electrophoresis, constant denaturant gel electrophoresis, and chemical cleavage analysis; (b) if no **sequence** variation is found in step (a), then **sequencing** the gene sample; and (c) determining the presence or absence of a **sequence** variation by analyzing the **sequence**(s) obtained in step (b) against a **reference sequence**.

5. The **method** of claim 1, 2, or 3 further comprising repeating the allele specific hybridization until a predetermined number of known **sequence** variations have been tested for.

6. The **method** of claim 5 wherein the allele specific hybridization assay is performed using a dot blot format.

7. The **method** of claim 5 wherein the allele specific hybridization assay is performed using a multiplex format.

8. The **method** of claim 1, 2, or 3 wherein the allele specific hybridization comprises testing for a predetermined number of **sequence** variations in a single step not requiring repetition.

9. The **method** of claim 8 wherein the allele specific

hybridization assay is performed using a reverse dot blot format, a MASDA format, or a chip array format.

10. The **method** of claim 1, 2, or 4 wherein the **sequence** variation locating assay is performed using a protein truncation assay.

11. The **method** of claim 1, 2, or 4 wherein the **sequence** variation locating assay is performed using a chemical cleavage assay, a heteroduplex analysis, a single strand conformation, polymorphism assay, a constant denaturing gel electrophoresis assay, or a denaturing gradient gel electrophoresis assay.

12. The **method** of claim 1, 2, 3, or 4 wherein **sequencing** is performed in only the forward or reverse direction.

13. The **method** of claim 1, 2, 3, or 4 wherein **sequencing** is performed in both the forward and reverse directions.

14. The **method** of claim 1, 2, 3, or 4 wherein **sequencing** comprises **sequencing** both exons and introns of the gene or parts thereof.

15. The **method** of claim 14 wherein all exons and all introns are **sequenced** from **end** to **end**.

16. The **method** of claim 1, 2, 3, or 4 wherein **sequencing** comprises **sequencing** only exons.

17. The **method** of claim 1, 2, 3, or 4 wherein **sequencing** comprises **sequencing** only intronic **sequences**.

18. The **method** of claim 1, 2, 3, or 4 wherein the gene sample is a human BRCA1 gene.

19. The **method** of claim 1, 2, 3, or 4 wherein the **reference sequence** is a coding **sequence**.

20. The **method** of claim 19 wherein the **reference sequence** is a BRCA1 coding **sequence**.

21. The **method** of claim 1, 2, 3, or 4 wherein the **reference sequence** is a genomic **sequence**.

22. The **method** of claim 21 wherein the **reference sequence** is a BRCA1 genomic **sequence**.

23. The **method** of claim 1, 2, 3, or 4 wherein the **reference sequence** is one or more exons of a gene of interest.

24. The **method** of claim 1, 2, or 3 wherein the predetermined **sequence** variation in step (a) is a known mutation.

25. The **method** of claim 1, 2, 3, or 4, wherein the **sequence** variation is a known mutation.

L7 ANSWER 12 OF 26 USPATFULL
AN 2000:4602 USPATFULL
TI Detection of differences in nucleic acids
IN Lishanski, Alla, San Jose, CA, United States
Kurn, Nurith, Palo Alto, CA, United States
Ullman, Edwin F., Atherton, CA, United States

PA Dade Behring Marburg GmbH, Marburg, Germany, Federal Republic of (non-U.S. corporation)
PI US 6013439 20000111
AI US 1996-771623 19961220 (8)
PRAI US 1996-12929 19960306 (60)
US 1995-9289 19951222 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Marschel, Ardin H.; Assistant Examiner: Tung, Joyce
LREP Leitereg, Theodore J.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 3375
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** for detecting the presence of a difference between two related nucleic acid **sequences**, said **method** comprising: (a) forming a complex comprising both of said nucleic acid **sequences** in double stranded form, wherein said complex comprises at least one pair of non-complementary strands and each of said non-complementary strands within said complex has a label, (b) subjecting said complex to strand exchange conditions wherein, if a difference between said two related nucleic acid **sequences** is present, strand exchange in said complex ceases and wherein, if no difference between said two related nucleic acid **sequences** is present, strand exchange in said complex continues until complete strand exchange occurs, and (c) detecting the association of said labels as part of said complex, the association thereof being related to the presence of said difference.

2. The **method** of claim 1 wherein said difference is a mutation.

3. The **method** of claim 1 wherein said nucleic acid **sequences** are DNA.

4. The **method** of claim 1 wherein said complex comprises a Holliday junction.

5. A **method** for detecting a mutation within a target nucleic acid **sequence**, said **method** comprising: (a) forming from said target **sequence** a tailed target partial duplex A' comprised of a duplex of two nucleic acid strands of said target **sequence**, a label and at one **end** of said duplex, two non-complementary oligonucleotides, one linked to each of said strands, (b) providing in combination said tailed target partial duplex A' and a tailed **reference** partial duplex B' lacking said mutation having a label as a part thereof, wherein said tailed **reference** partial duplex B' is comprised of two nucleic acid strands, each of said strands being complementary, respectively, to a strand in said tailed target partial duplex A' but for the possible presence of a mutation and wherein said labels are present in non-complementary strands of said tailed target and tailed **reference** partial duplexes, respectively, (c) subjecting said combination to strand exchange conditions wherein, if a mutation is present, strand exchange in said complex ceases and wherein, if no mutation is present, strand exchange in said complex continues until complete strand exchange occurs, and (d) detecting, by means of said labels, the formation of a complex between said tailed partial duplexes, the formation thereof being directly related to the presence of said mutation.

portion of genome

6. The **method** of claim 5 wherein said target nucleic acid **sequence** is DNA.

7. The **method** of claim 5 wherein said tailed **reference** partial duplex B' is provided in said combination by forming said tailed

reference partial duplex B' in the same reaction medium as that used for step (a).

8. The **method** of claim 7 wherein forming said tailed target partial duplex A' and said tailed **reference** partial duplex B' is carried out simultaneously.

9. The **method** of claim 5 wherein said labels are independently selected from the group consisting of oligonucleotides, enzymes, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, small organic molecules and solid surfaces.

10. The **method** of claim 6 wherein said non-complementary oligonucleotides each have from 15 to 60 nucleotides.

11. A **method** for detecting a mutation in a nucleic acid, said **method** comprising: (a) producing, from a target nucleic acid **sequence** suspected of having a mutation, a partial duplex A' comprising a fully complementary double stranded nucleic acid **sequence** containing said target nucleic acid **sequence** wherein one strand has at its 5'-**end** a portion A1 that does not hybridize with a corresponding portion A2 at the 3'-**end** of the other strand, wherein one of said strands of said partial duplex A' comprises a label, (b) producing, from a **reference** nucleic acid **sequence** that corresponds to said target nucleic acid **sequence** of step (a) except for said mutation, a partial duplex B' comprising said double stranded nucleic acid **sequence** lacking said mutation wherein the strand that is complementary, except for said portion A1, to the strand of said partial duplex A' comprising said portion A1 has at its 5'-**end** a portion B1 that is complementary with said A2 and the other strand has at its 3'-**end** a portion B2 that is complementary with said A1, wherein one of said strands of said partial duplex B' comprises a label, said strand comprising said label being unable to hybridize directly to said strand of said partial duplex A' that comprises a label, (c) subjecting said partial duplexes A' and B' strand exchange to conditions that permit said duplexes to hybridize to each other wherein, if said target nucleic acid **sequence** having said mutation is present, a stable complex is formed comprising said partial duplex A' and said partial duplex B' and wherein, if said target nucleic acid **sequence** having said mutation is not present, strand exchange in said complex continues until complete strand exchange occurs, and (d) determining whether said stable complex is formed, the presence thereof indicating the presence of said nucleic acid having said mutation.

they do have 1) seq. of int. 2) ref. seq.

12. The **method** of claim 11 wherein said labels are independently selected from the group consisting of oligonucleotides, enzymes, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, small organic molecules, polynucleotide **sequences** and solid surfaces.

13. The **method** of claim 11 wherein steps (a) and (b) are carried out simultaneously in the same reaction medium.

14. The **method** of claim 11 wherein said A1 and said A2 each have from 15 to 60 nucleotides.

15. The **method** of claim 11 wherein said nucleic acid is DNA.

16. A **method** for detecting a target nucleic acid **sequence**, said **method** comprising: (a) forming from said target nucleic acid **sequence** a tailed target partial duplex A' comprised of a duplex of two nucleic acid strands of said target nucleic acid **sequence**, a label, and at one **end** of said duplex, two non-complementary oligonucleotides, one linked to each of said strands, (b) providing in combination (i) said tailed target partial duplex A' and (ii) a tailed **reference** partial

duplex B' comprising a duplex of two nucleic acid strands of a **sequence** different than said target nucleic acid **sequence**, a label and, at one **end** of said duplex, two oligonucleotides that are complementary to said two non-complementary oligonucleotides of said tailed target partial duplex A", one linked to each of said strands of said tailed **reference** partial duplex B", wherein said labels are on non-complementary strands, (c) subjecting said combination to strand exchange conditions wherein, if said target nucleic acid **sequence** is present, strand exchange in said complex ceases and wherein, if no target nucleic acid **sequence** is present, strand exchange in said complex continues until complete strand exchange occurs, and (d) detecting, by means of said labels, the formation of a complex between said partial duplexes A' and B', the formation thereof being directly related to the presence of said target nucleic acid **sequence**.

17. The **method** of claim 16 wherein said target and said **reference** nucleic acid **sequences** are identical but for a mutation.

18. The **method** of claim 16 for detecting a target nucleic acid **sequence** that does not contain a mutation.

19. A **method** for detecting a target nucleic acid **sequence**, said **method** comprising: (a) producing, from a target nucleic acid **sequence**, a partial duplex A' comprising a fully complementary double stranded nucleic acid **sequence** containing said target nucleic acid **sequence** wherein one strand has at its 5-**end** a portion A1 that does not hybridize with a corresponding portion A2 at the 3'-**end** of the other strand, wherein one of said strands of said partial duplex A' comprises a label, (b) producing, from a **reference** nucleic acid **sequence**, a partial duplex B' comprising a duplex of two nucleic acid strands different from said target nucleic acid **sequence**, wherein the strand that is complementary, except for said portion A1, to the strand of said partial duplex A' comprising said portion A1 has at its 5'-**end** a portion B1 that is complementary with said A2 and the other strand has at its 3'-**end** a portion B2 that is complementary with said A1, wherein one of said strands of said partial duplex B' comprises a label, said strand comprising said label being unable to hybridize directly to said strand of said partial duplex A' that comprises a label, (c) subjecting said partial duplexes A' and B' strand exchange to conditions that permit said duplexes to hybridize to each other to form a quadramolecular complex wherein, if said target nucleic acid **sequence** is present, strand exchange in said complex ceases and wherein, if no target nucleic acid **sequence** is present, strand exchange in said complex continues until complete strand exchange occurs, and (d) determining whether said complex is formed, the presence thereof indicating the presence of said target nucleic acid **sequence**.

20. The **method** of claim 19 wherein said target and said **reference** nucleic acid **sequences** are identical but for a mutation.

21. The **method** of claim 19 for detecting a target nucleic acid **sequence** that does not contain a mutation.

L7 ANSWER 14 OF 26 USPATFULL
AN 1999:85221 USPATFULL
TI Methods for the detection of loss of heterozygosity
IN Lapidus, Stanley N., Bedford, NH, United States
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PI US 5928870 19990727
AI US 1997-876857 19970616 (8)

DT Utility
FS Granted
EXNAM Primary Examiner: Houtteman, Scott W.
LREP Testa, Hurwitz & Thibeault,LLP
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1069
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** for detecting the presence of a mutant nucleic acid in a sample population, comprising the steps of: a) introducing a first radionucleotide to a sample population suspected to contain a subpopulation of a nucleic acid mutant, wherein said first radionucleotide hybridizes to a first wild-type nucleic acid target, a subpopulation of which suspected to be mutated in the sample; b) introducing a second radionucleotide to the sample, wherein said second radionucleotide hybridizes to a second wild-type nucleic acid target in the sample c) washing said sample to remove unhybridized first and second radionucleotides; d) determining a number X of radioactive decay events associated with said first radionucleotide; e) determining a number Y of radioactive decay events associated with said second radionucleotide; f) determining whether a difference exists between number X and number Y, the presence of a statistically-significant difference being indicative of the presence of a mutation in said sample.

2. The **method** of claim 1 wherein said first radionucleotide is capable of hybridizing to a nucleic acid in the sample that is suspected to be mutated in cancer or precancer; and said second radionucleotide is capable of hybridizing to a nucleic acid in the sample that is not mutated in cancer or precancer.

3. The **method** of claim 1 wherein said first radionucleotide is capable of hybridizing to a portion of the maternal allele at a genetic locus; and said second radionucleotide is capable of hybridizing to a portion of the paternal allele at said locus.

4. The **method** of claim 1 further comprising the step of isolating said first radionucleotide specifically bound to a first target nucleic acid, and said second radionucleotide specifically bound to a second target nucleic acid.

5. The **method** of claim 4 wherein said isolating step is selected from the group consisting of gel electrophoresis, chromatography, and mass spectrometry.

6. The **method** of claim 4 wherein said number X is correlated with a number X1 of molecules of said first nucleic acid, and said number Y is correlated with a number Y1 of molecules of said second nucleic acid.

7. The **method** of claim 1 wherein at least one of said first and second radionucleotides is a chain **terminator** nucleotide.

8. The **method** of claim 1 wherein at least one of said first and second radionucleotides is an oligonucleotide.

9. The **method** of claim 1 wherein said radionucleotides are labeled with an isotope selected from the group consisting of 32P, 33P, 35S, 125I and 14C.

10. The **method** of claim 1 wherein each of said first and second radionucleotides are labeled with a different isotope.

11. The **method** of claim 10 wherein said numbers X and Y are determined by coincidence counting.

12. A **method** for determining the number of molecules of a nucleic acid comprising the steps of: a) exposing a sample to a plurality of first radionucleotides; b) isolating radionucleotides specifically bound to first target nucleic acid molecules; c) determining a number of radioactive decay events associated with the radionucleotides of step b); d) calculating a number of molecules of said **sequence** as equivalent to said number of radioactive decay events.

13. A **method** for detecting the presence of a mutation in a nucleic acid, comprising the steps of: a) exposing a sample to a plurality of a oligonucleotide; b) performing a primer extension reaction in the presence of a plurality of a chain **terminating** nucleotide, to generate extension products of said oligonucleotide; c) determining the size of the extension products, the presence of extension products of different sizes being indicative of the presence of a mutation.

14. The **method** of claim 13 wherein said oligonucleotide is capable of hybridizing to a member selected from the group consisting of a maternal allele and a paternal allele of the same genetic locus.

15. The **method** of claim 13 wherein said oligonucleotide is labeled.

16. The **method** of claim 13 wherein said **terminating** nucleotide is labeled.

17. The **method** of claims 15 or 16 wherein said label is a radioactive isotope.

18. The **method** of claim 13 wherein said extension reaction is performed in the presence of at least two differentially labeled chain **terminating** nucleotides.

19. A **method** for detecting loss of heterozygosity in a nucleic acid, comprising the steps of: a) contacting a sample with a radionucleotide; b) isolating a nucleic acid specifically bound to said radionucleotide; c) determining a number of radioactive decay events associated with said nucleic acid; d) comparing said number to a **reference** number, wherein a statistically significant difference between said number and said **reference** number is indicative of loss of heterozygosity.

20. The **method** of claim 1, wherein said sample comprises cellular material from a population of patients.

21. The **method** of claim 20, wherein said population of patients is healthy.

22. The **method** of claim 20, wherein said population of patients has a disease suspected to be associated with said mutant nucleic acid.

23. The **method** of claim 20, wherein said disease is cancer.

24. The **method** of claim 1, wherein said mutant nucleic acid is an allelic variant.

25. The **method** of claim 24, wherein said variant is a single nucleotide polymorphism.

L7 ANSWER 20 OF 26 USPATFULL
AN 96:9349 USPATFULL
TI Methods for detecting and assaying nucleic acid sequences

IN Nilsen, Thor W., Glen Mills, PA, United States
Prensky, Wolf, Fairlawn, NJ, United States
PA PolyProbe, Inc., Media, PA, United States (U.S. corporation)
PI US 5487973 19960130
AI US 1992-963107 19921019 (7)
RLI Division of Ser. No. US 1986-906222, filed on 10 Sep 1986, now patented, Pat. No. US 5175270
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Marschel, Ardin H.
LREP Curtis Morris & Safford, Evans, Barry
CLMN Number of Claims: 27
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1545
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** of determining the presence of a specific **sequence** of nucleotides in a nucleic acid target molecule in a sample by detecting the presence of a hybrid thereof, and not detecting the hybrid in the absence thereof which comprises; (A) providing a reagent for the detection and assay of the **sequence** of nucleotides in the nucleic acid target molecule, which comprises; (a) a plurality of molecules, each of which comprises a first partially double-stranded polynucleotide having a structure comprising a first molecule **end**, a second molecule **end** and a double-stranded body portion intermediate of the first and second **ends** thereof; said first and second **ends** thereof each having at least one of first and second arms consisting of a single strand of polynucleotide; said single strands being hybridizable with a pre-determined **sequence** of nucleotides in a nucleic acid; the first and second arms of each of said first and second **ends** being non-hybridizable with each other; (b) a plurality of molecules, each of which comprises a second partially double-stranded polynucleotide having a structure comprising a first molecule **end**, a second molecule **end** and a double-stranded body portion intermediate of the first and second **ends** thereof; said first and second **ends** thereof each having at least one of first and second arms thereof consisting of a single strand of polynucleotide; said single strands being hybridizable with a pre-determined **sequence** of nucleotides in a nucleic acid; the first and second arms of each of said first and second **ends** being non-hybridizable with each other; said plurality of molecules of the first polynucleotide and the second polynucleotide being joined through annealing of one or more arms thereof, to form a matrix; and at least one non-annealed arm of said plurality of first and second polynucleotide molecules located on the surface of the matrix being free to hybridize and capable of hybridizing with the **sequence** of nucleotides in the nucleic acid target molecule, the presence of which is to be determined; (B) contacting the reagent with the sample under hybridization conditions such that the at least one non-annealed arm of the matrix can hybridize with **sequence** of nucleotides in the nucleic acid target molecule, only if present in the sample to form the hybrid thereof; and (C) detecting the presence of the hybrid, if present, as indicative of the presence of the specific **sequence** of nucleotides in a nucleic acid target molecule.

2. The **method** of claim 1 wherein the first and second polynucleotides are molecules of DNA.

3. The **method** of claim 1 wherein the reagent bears a detectable, signal generating marker.

4. The **method** of claim 1 wherein the nucleic acid target molecule is one associated with a bacterial or a viral pathogen.

5. The **method** of claim 1 wherein the hybrid is bound to a

water-insoluble support surface.

6. A **method** of detecting and assaying for the HIV-I virus in a sample by determining the presence in the sample of a **sequence** of nucleotides in a nucleic acid associated with HIV-I virus, by detecting a hybrid thereof, which comprises; (A) providing a reagent, which comprises; (a) a plurality of molecules, each of which comprises a first partially double-stranded polynucleotide having a structure comprising a first molecule **end**, a second molecule **end** and a double-stranded body portion intermediate of the first and second **ends** thereof; said first and second **ends** each having at least one of first and second arms consisting of a single strand of polynucleotide; said single strands being hybridizable with a pre-determined **sequence** of nucleotides in a nucleic acid; the first and second arms of each of said first and second **ends** being non-hybridizable with each other; (b) a plurality of molecules, each of which comprises a second partially double-stranded polynucleotide having a structure comprising a first molecule **end**, a second molecule **end** and a double-stranded body portion intermediate of the first and second **ends** thereof; said first and second **ends** thereof each having at least one of first and second arms thereof consisting of a single strand of polynucleotide; said single strands being hybridizable with a pre-determined **sequence** of nucleotides in a nucleic acid; the first and second arms of each of said first and second **ends** being non-hybridizable with each other; said plurality of molecules of the first polynucleotide and the second polynucleotide being joined together through annealing of one or more arms thereof, to form a matrix; and at least one non-annealed arm of said plurality of first and second polynucleotide molecules located on the outer surface of the matrix being free to hybridize and capable Of hybridizing with the **sequence** of nucleotides in the nucleic acid associated with the HIV-I virus, the presence of which is to be determined; (B) contacting the reagent with the sample under hybridization conditions such that the at least one non-annealed arm of the matrix can hybridize with the **sequence** of nucleotides in the nucleic acid associated with the HIV-I virus, only if present in the sample, to form a hybrid thereof; and (C) detecting the presence of the hybrid, if present, as indicative of the presence in a sample of a **sequence** of nucleotides in a nucleic acid associated with HIF-I virus.

7. The **method** of claim 6 wherein the reagent bears a detectable, signal generating marker.

8. The **method** of claim 6 wherein the hybrid is bound to a water-insoluble support surface.

9. A **method** of detecting or quantitating a specific **sequence** of nucleotides in an analyte of interest by detecting the presence of or quantitating the amount present of a hybrid thereof and not detecting the presence or quantitating the amount of the hybrid in the absence thereof, which **method** comprises: (a) forming a mixture of (i) a sample which may contain said **sequence** of nucleotides in the analyte of interest, and (ii) a composition containing a plurality of polynucleotides each having at at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides, at least one of the non-bonded single stranded hybridization regions of said composition being complementary to the **sequence** of nucleotides in the analyte of interest; (b) allowing said composition and said **sequence** of nucleotides in the analyte of interest, only if present in the sample, to hybridize and form the hybrid; and (c) detecting the presence of or quantitating the

amount present of the hybrid, if present, as indicative of the presence or quantity, respectively, of a specific **sequence** of nucleotides in the analyte of interest.

10. A **method** according to claim 9, wherein the composition is bound to a non-nucleic acid support.

11. A **method** according to claim 9, wherein the composition further contains a label and said label is detected or quantitated in said hybrid.

12. A **method** according to claim 9, wherein the analyte of interest is a **sequence** of nucleotides of a pathogen.

13. A **method** according to claim 9, wherein the pathogen is a HIV-I virus.

14. A **method** of detecting or quantitating a specific **sequence** of nucleotides in an analyte of interest by detecting the presence of or quantitating the amount present of a hybrid complex thereof and not detecting the presence or quantitating the amount of the hybrid complex in the absence thereof, which **method** comprises: (a) forming a first mixture of (i) a simple which may contain said **sequence** of nucleotides in the analyte of interest and (ii) a first composition containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides; at least one of the non-bonded single stranded hybridization regions can be capable of hybridizing with the **sequence** or a portion of the **sequence** of nucleotides in the analyte of interest; at least one of the non-bonded single-stranded hybridization regions can be capable of hybridizing with non-bonded single stranded hybridization regions of a second composition; and at least one of the non-bonded single stranded hybridization regions is capable of hybridizing either to the **sequence** or portion of the **sequence** of nucleotides in the analyte of interest or to the non-bonded single stranded hybridization regions of the second composition; (b) optionally allowing said first composition and said **sequence** of nucleotides in the analyte of interest to hybridize and form a first hybrid complex only if said **sequence** of nucleotides in the analyte of interest is present in the sample and if the at least one non-bonded single stranded hybridization regions of the first composition is capable of hybridizing with the **sequence** or a portion of the **sequence** of nucleotides in the analyte of interest; (c) forming a second mixture of (i) said first mixture and (ii) the second composition, said second composition containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides, at least one of the non-bonded single stranded hybridization regions of said second composition being complementary to at least one of the non-bonded single stranded hybridization regions of said first composition or to at least a portion of the **sequence** of nucleotides in the analyte of interest; and (d) subjecting said second mixture to hybridization conditions to form a second hybrid complex only if the **sequence** of nucleotides in the analyte of interest is present, wherein said second hybrid complex comprises: (i)

the first and second compositions each hybridization bonded to the analyte of interest, (ii) the first and second compositions hybridization bonded to each other and the second composition hybridization bonded to the analyte of interest, or (iii) the first and second compositions hybridization bonded to each other and the first composition hybridization bonded to the analyte of interest; and (e) detecting the presence of or quantitating the amount present of said second hybrid complex and thus of the specific **sequence** of nucleotides in the analyte of interest.

15. A **method** according to claim 14, wherein at least one of the first and second compositions is bound to a non-nucleic acid support.

16. A **method** according to claim 14, wherein at least one of the first and second compositions contains a label and said label is detected in said second hybrid complex.

17. A **method** according to claim 14, wherein the analyte of interest is a **sequence** of nucleotides of a pathogen.

18. A **method** according to claim 17, wherein the pathogen is a HIV-I virus.

19. A **method** of detecting or quantifying a specific **sequence** of nucleotides in an analyte of interest by detecting the presence of or quantitating the amount present of a hybrid complex thereof and not detecting the presence or quantitating amount of the hybrid complex in the absence thereof, which **method** comprises: (a) forming a mixture of (i) a sample which may contain said **sequence** of nucleotides in the analyte of interest and (ii) first and second compositions containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides, at least one of the non-bonded single stranded hybridization regions of said first and of said second compositions being complementary to different parts respectively of the **sequence** of nucleotides in the analyte of interest and to non-bonded single stranded hybridization regions of a third composition; (b) allowing said first and second compositions and said **sequence** of nucleotides in the analyte of interest to hybridize forming a first hybrid complex only if said **sequence** of nucleotides in the analyte of interest is present in the sample; (c) forming a mixture of (i) said first hybrid complex and (ii) the third composition, said third composition containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides, at least one of the non-bonded single stranded hybridization regions of said third composition being complementary to at least one of the non-bonded single stranded hybridization regions of at least one of said first and second compositions; (d) allowing said first hybrid complex and said third composition to hybridize forming a second hybrid complex; and (e) detecting the presence of or quantitating the amount present of said second hybrid complex and thus of the specific **sequence** of the nucleotides in the analyte of interest.

20. A **method** according to claim 19, wherein at least one of the first, second and third compositions is bound to a non-nucleic acid support.

21. A **method** according to claim 19, wherein at least one of the first, second and third compositions contains a label and said label is detected in said second hybrid complex.

22. A **method** according to claim 19, wherein the analyte of interest is a **sequence** of nucleotides of a pathogen.

23. A **method** according to claim 22, wherein the pathogen is a HIV-I virus.

24. A **method** of determining the concentration of a specific **sequence** of nucleotides in an analyte of interest in a composition by **reference** to sizes of compositions in hybrid complexes thereof and not determining the concentration of the **sequence** of nucleotides in the analyte of interest in the composition in the absence of hybrid complexes thereof, which **method** comprises: (a) contacting (i) a plurality of compositions each containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, and wherein a plurality of the single stranded hybridization regions are not bonded to any other polynucleotides, at least one of the non-bonded single stranded hybridization regions of each of said compositions being complementary to the **sequence** of nucleotides in the analyte of interest, and each said composition being a different size, and (ii) a solid support containing bound **sequence** of nucleotides in the analyte of interest, under conditions conducive to hybridization of said compositions and said analyte of interest to form hybrid complexes on the solid support, only if said **sequence** of nucleotides in the analyte of interest is present in the sample; (b) washing the solid support to remove unhybridized compositions; (c) detecting said hybrid complexes if present, on the solid support; and (d) determining the concentration of said analyte of interest by **reference** to the sizes of the compositions of said hybrid complexes on the solid support.

25. A **method** of detecting or quantitating a specific **sequence** of nucleotides in an analyte of interest by detecting the presence of or quantitating the amount of hybrid thereof and not detecting the presence or quantitating amount of the hybrid in the absence thereof, which **method** comprises (a) contacting (i) a single which may contain said **sequence** of nucleotides in the analyte of interest and (ii) a composition containing a plurality of polynucleotides each having at least three single stranded hybridization regions, wherein any one polynucleotide is bonded by hybridization or hybridization and covalent bonding to any other polynucleotide at at least one such hybridization region, and when bonded to more than one such hybridization region of the same polynucleotide there is an intermediate region where the two polynucleotides are not bonded, said composition bound to a solid support, and, at least one of said single stranded hybridization regions capable of hybridizing under conditions conducive to hybridization with said **sequence** of nucleotides, only if present in the analyte of interest to form a hybrid; and (b) detecting the presence of or quantitating the amount of the hybrid, if present, as indicative the presence of quantity, respectively, of the specific **sequence** of nucleotides in the analyte of interest.

26. A **method** according to claim 25, wherein the analyte of interest is a **sequence** of nucleotides of a pathogen.

27. A **method** according to claim 25, wherein the pathogen is a HIV-I virus.

L7 ANSWER 22 OF 26 USPATFULL
AN 94:15668 USPATFULL
TI Instrument and method for the sequencing of **genome**
IN Beavis, Ronald C., New York, NY, United States
Chait, Brian T., New York, NY, United States
PA The Rockefeller University, New York, NY, United States (U.S. corporation)
PI US 5288644 19940222
AI US 1992-957688 19921113 (7)
RLI Continuation of Ser. No. US 1990-504643, filed on 4 Apr 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Redding, David
LREP Browning, Bushman, Anderson & Brookhart
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 448
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A **method** of DNA **sequencing** of the **genome** by detecting the mass weights of DNA fragments comprising the steps of: (a) producing a piece of DNA of unknown **sequence**; (b) performing four different base-specific reactions on the piece of DNA to produce four different DNA sets containing DNA fragments each DNA set having a common origin and **terminating** at a particular base along the unknown **sequence**; (c) selecting a solid matrix having a strong absorption band at the wavelength of light produced by a laser and placing one of the DNA sets on the matrix, the strong absorption band of the matrix having a longer wavelength than the absorption bands for the DNA set; (d) without separation of the DNA fragments by electrophoresis or other separation **methods** and without labeling the DNA fragments with radioactive, fluorescent or other labeling means, striking the DNA fragments on the solid matrix within one of the DNA sets with a series of laser pulses from the laser to desorb ions of the DNA fragments and produce ionized DNA fragments; (e) detecting the mass weights of the ionized DNA fragments by a time of flight mass spectrometer; and (f) repeating steps (d) and (e) for the other DNA sets to determine the **sequence** of bases in the DNA.

2. The **method** as defined in claim 1, wherein each different set of DNA fragments are mixed with the selected solid matrix.

3. The **method** as defined in claim 1, wherein the surface of the solid matrix has a plurality of discrete spots formed thereon, each discrete spot containing one of the different sets of DNA fragments; step (e) including applying a vacuum and an electric field within the mass spectrometer; and the step of detecting the mass weights includes detecting the molecular weight of the DNA fragments contained in each spot.

4. The **method** as defined in claim 3, wherein said plurality of discrete spots on the surface of the solid matrix are each at a fixed location with respect to a **reference** point on the surface.

5. The **method** as defined in claim 1, wherein the step of detecting the mass weights further comprises: determining the absolute mass difference between the detected molecular weight of weight of a peak of one of the sets of DNA fragments compared to a peak of another of the sets of DNA fragments; and correcting the **sequence** of the bases in the DNA in response to the determined absolute mass differences.

6. A **method** of DNA **sequencing** of the **genome**, comprising the steps of: (a) producing a piece of DNA of unknown **sequence**; (b) performing at least four different reactions on the piece of DNA to produce at least four different sets containing DNA fragments each having a common origin and **terminating** at a particular base along the unknown **sequence**; (c) striking the DNA fragments within one of the different sets with a series of laser pulses to desorb ions of the DNA fragments; (d) detecting the mass weights of the ionized fragments within the one set by a time of flight mass spectrometer; and (e) repeating steps (c) and (d) for other of the different sets of DNA fragments to determine the **sequence** of bases in the DNA.

7. The **method** as defined in claim 6, wherein the surface of the solid matrix has a plurality of discrete spots formed thereon, each discrete spot containing one of the different sets of DNA fragments; step (e) including applying a vacuum and an electric field within the mass spectrometer; and the step of detecting the mass weights includes detecting the molecular weight of the DNA fragments contained in each spot.

8. The **method** as defined in claim 7, wherein said plurality of discrete spots on the surface of the solid matrix are each at a fixed location with respect to a **reference** point on the surface.

d bib,abs 28, 30

L14 ANSWER 28 OF 32 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-466001 [40] WPIDS
DNC C2000-140396
TI Identification of novel nucleic acid **sequences** used to identify variations within the human **genome** including in diseased tissues.
DC B04 D16
IN MCKENNA, M; PREDKI, P; ROTHBERG, J M; SHIMKETS, R A; WINDEMUTH, A
PA (CURA-N) CURAGEN CORP
CYC 90
PI WO 2000040757 A2 20000713 (200040)* EN 45p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000029612 A 20000724 (200052)
ADT WO 2000040757 A2 WO 2000-US402 20000107; AU 2000029612 A AU 2000-29612 20000107
FDT AU 2000029612 A Based on WO 200040757
PRAI US 1999-417386 19991013; US 1999-115109 19990108
AN 2000-466001 [40] WPIDS
AB WO 200040757 A UPAB: 20000823
NOVELTY - Screening a population of nucleic acids for a novel **sequence** comprises partitioning the nucleic acid population into one or more subpopulations, identifying a first nucleic acid (I) in the subpopulation and comparing (I) to a **reference sequence** (s) where the absence of (I) in the **reference sequence** (s) indicates (I) is a novel **sequence**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a **method** for equalizing the representation of nucleic acids in a population of nucleic acids comprising providing a population of nucleic acid **sequences** with first (II) and second (II) nucleic acid **sequences** with distinct **sequences** with (II) present at a higher level in the population, partitioning the population into one or more subpopulations and comparing the level of (II) to the level of (III) in the subpopulations where a lower level of (II) relative to (III) indicates the representation of (II) and (III) are normalized;

(2) a **method** for producing a population of nucleic acid molecules enriched for 5' regions of mRNA molecules comprising providing a population of RNA molecules which includes RNA molecules with a 5' **terminal** Gppp cap structure and a 5' **terminal** phosphate group, contacting the RNA population with a phosphatase under conditions which will remove the 5' **terminal** phosphate group leaving the 5' **terminal** Gppp cap structure intact, inactivating the phosphatase contacting the population of RNA of molecules with a pyrophosphatase under conditions which remove the 5' **terminal** Gppp and form a 5' phosphate group, annealing an oligonucleotide in the presence of an RNA ligase to form a hybrid molecule and forming a cDNA from the oligonucleotide;

(3) a **method** (M1) of identifying an RNA **sequence** in a sample comprising synthesizing cDNA copies of RNA species to form a cDNA sample, determining the size of one or more the cDNA molecules in the cDNA sample, comparing the size of the sample with the size of a **reference** nucleic acid and identifying the cDNA **sequence** ; and

(4) a **method** of identifying an RNA **sequence** in a population of RNA **sequences** comprising removing 5' **terminal** pppG to form a population of RNAs with **terminal** 5' phosphate groups, synthesizing cDNAs from the population, digesting the cDNAs with at least one restriction enzyme, ligating an adapter molecule to the digested cDNA molecules, amplifying and then identifying the

molecules produced and comparing the amplified molecules to one or more **reference** nucleic acids.

USE - The **methods** are used for identifying genes in an organism of interest e.g. human especially for genes which are transcribed at low levels or which generate low levels of steady state transcripts, to identify variations within the human **genome** e.g. single nucleotide polymorphisms, identify differences between normal and diseased tissue and to analyze differential gene expression in different tissues and/or species.

ADVANTAGE - The **methods** eliminate or minimize redundant characterization of identical nucleic acid **sequences** in a population of nucleic acids.

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L14 ANSWER 30 OF 32 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-237668 [20] WPIDS
DNC C2000-072353
TI Determining presence and identity of polynucleotide **sequence** variations between two polynucleotides useful for diagnosing and treating specific disease uses amplification reactions.
DC B04 D16
IN DAWSON, E P; PHILLIPS, J A
PA (BIOV-N) BIOVENTURES INC; (UYVA-N) UNIV VANDERBILT
CYC 88
PI WO 2000011221 A1 20000302 (200020)* EN 26p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9956813 A 20000314 (200031)
EP 1105537 A1 20010613 (200134) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
AU 737087 B 20010809 (200152)
ADT WO 2000011221 A1 WO 1999-US18965 19990819; AU 9956813 A AU 1999-56813 19990819; EP 1105537 A1 EP 1999-943782 19990819, WO 1999-US18965 19990819; AU 737087 B AU 1999-56813 19990819
FDT AU 9956813 A Based on WO 200011221; EP 1105537 A1 Based on WO 200011221; AU 737087 B Previous Publ. AU 9956813, Based on WO 200011221
PRAI US 1998-97136 19980819
AN 2000-237668 [20] WPIDS
AB WO 200011221 A UPAB: 20000426

09/719130 → 09/994119 = US 6322988

NOVELTY - A **method** (I) for determining the presence of a nucleotide **sequence** variation between two polynucleotides, is new and comprises subjecting a region containing variation in a first polynucleotide (A) to amplification, producing labeled polynucleotide fragments from both strands of the amplified products by a fragment producing reaction and comparing the location and identity to a second polynucleotide (B).

DETAILED DESCRIPTION - A **methods** (I) for determining the presence and identity of a variation in a nucleotide **sequence** between two polynucleotides, is new and comprises:

(a) selecting a region of a first polynucleotide (A) potentially containing the variation;

(b) subjecting the selected region to a template producing amplification reaction to produce double stranded polynucleotide templates which include the selected region;

(c) producing a family of labeled, linear polynucleotide fragments from both strands of the template simultaneously by a fragment producing reaction using a set of primers, where each of the fragments are **terminated** at the 3' **end** and the family includes at least one fragment **terminating** at each possible base, represented by the **terminator**, of the portion of the strands that are flanked by the primers;

(d) determining the location and identity of at least some of the

bases in the selected region of (A) using the labels in the fragments; and

(e) comparing this with the location and identity of a variation in a **corresponding** region of a second polynucleotide (B).

USE - (I) is used to determine the presence and identity of a variation in a nucleotide **sequence** between two polynucleotides (claimed), for diagnosing, treating or preventing specific diseases in individuals. (I) is also useful for understanding the relation ship between **genome** variations and environmental factors in the pathogenesis of diseases and prevalence of conditions.

ADVANTAGE - The **method** is simple and can simultaneously identify and quantify known and unknown variations accurately and determine the locations, identities and frequencies of all variations between two populations of polynucleotides. The **method** also determines two or more genetic variations residing on the same or different **alleles** in an individual, and can be used to determine the frequency of occurrence of the variation in a population.

Dwg.0/0

-- (FILE 'HOME' ENTERED AT 14:24:1[redacted]ON 03 OCT 2001)

FILE 'USPATFULL' ENTERED AT 14:24:34 ON 03 OCT 2001
L1 0 S (END SEQUENCE PROFILING)
L2 11 S (GENOME (8A) REFERENCE)/CLM
L3 4 S (GENOME (8A) REFERENCE)/CLM NOT AMPLIF?/CLM

=> d bib,hit 1-3

L3 ANSWER 1 OF 4 USPATFULL
AN 2000:153229 USPATFULL
TI High density array fabrication and readout method for a fiber optic biosensor
IN Pinkel, Daniel, Walnut Creek, CA, United States
Gray, Joe, San Francisco, CA, United States
Albertson, Donna G., Lafayette, CA, United States
PA The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
Medical Research Council, London, United Kingdom (non-U.S. corporation)
PI US 6146593 20001114
AI US 1997-899000 19970724 (8)
RLI Division of Ser. No. US 1995-448043, filed on 23 May 1995, now patented, Pat. No. US 5690894
DT Utility
FS Granted
EXNAM Primary Examiner: Fredman, Jeffrey
LREP Skjerven, Morrill, MacPherson LLP, Haliday, Emily M.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 1396
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
10. The method of claim 1, wherein the first collection of labeled nucleic acid molecules is from a test genome and the second collection of labeled nucleic acid molecules is from a normal **reference genome**.

L3 ANSWER 2 OF 4 USPATFULL
AN 1998:134801 USPATFULL
TI Comparative fluorescence hybridization to nucleic acid arrays
IN Pinkel, Daniel, Walnut Creek, CA, United States
Albertson, Donna, Cambridge, United Kingdom
Gray, Joe W., San Francisco, CA, United States
PA The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
The Medical Research Council, Cambridge, England (non-U.S. corporation)
PI US 5830645 19981103
AI US 1994-353018 19941209 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Townsend and Townsend and Crew
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 897
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
14. The method of claim 1, wherein the first collection of labeled nucleic acids is from a test genome and the second collection of labeled nucleic acids is from a normal **reference genome**.

L3 ANSWER 3 OF 4 USPATFULL

AN 1998:108282 USPATFULL
TI Identification of homologous gene products across species boundaries or of a single species
IN Humphery-Smith, Ian, Sydney, Australia
PA The University of Sydney, New South Wales, Australia (non-U.S. corporation)
PI US 5804449 19980908
WO 9610175 19960404
AI US 1997-809217 19970519 (8)
WO 1995-AU641 19950828
19970519 PCT 371 date
19970519 PCT 102(e) date
PRAI AU 1994-8456 19940928
DT Utility
FS Granted
EXNAM Primary Examiner: Scheiner, Toni R.
LREP Kirschstein et al.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 774
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

2. A method for determining whether a test gene product is homologous with a **reference** gene product produced by an organism having a **genome** which differs significantly in size from the size of the genome of the organism producing the test gene product, which method comprises the steps of: 1) comparing the M.sub.r of the test gene product with the M.sub.r of the reference gene product, 2) if the M.sub.r of the test gene product differs from the M.sub.r of the reference gene product by not more than the greater of 10 kD or 10% of the M.sub.r of the reference gene product, comparing the silver stained colour of the test gene product with the silver stained colour of the reference gene product, 3) if the silver stained colour of the test gene product is within 20% of shade units of the colour of the reference gene product, comparing the level of expression of the test gene product with the level of expression of the reference gene product, 4) if the absolute level of expression of the test gene product differs from the absolute level of expression of the reference gene product by not more than 40%, comparing the pI of the test gene product with the pI of the reference gene product; 5) determining whether the pI of the test gene product differs from the pI of the reference gene product by not more than 4 pH units, and if all the criteria specified at steps 2) to 4) are met, determining that the test gene product is homologous to the reference gene product.